

73. The method of claim 55, wherein the functional domain comprises an epitope tag.

74. The method of claim 62, wherein the functional domain comprises an epitope tag.--

Amendments to Specification:

The specification has been amended to correct typographical errors. Support of these amendments can be found as follows:

Amendment	Support
col. 9, line 15	page 13, last line to first line of page 14 of PCT/GB95/01949
col. 12, line 53	page 20, line 11 of PCT/GB95/01949
col. 20, line 15	page 30, third line from the bottom of PCT/GB95/01949
col. 20, line 17	Medline reference
col. 22, line 4	page 33, second line from the bottom of PCT/GB95/01949
col. 29, line 63	Rule 312 amendment (filed July 27, 1998) deleting certain Figures.
col. 30, line 4	page 46, last line of PCT/GB95/01949

Applicants note that the application from which U.S. Patent No. 6,007,988 issued was a 371 filing of PCT/GB95/01949.

Added claims 42 to 74

Added claims 42 to 53 are composition claims and added claims 54 to 74 are method claims. Exemplary support for these claims is shown in the following table:

Claim(s)	Exemplary Support
42	col. 8, lines 17-23; col. 4, line 48 to col. 5 line 8
43, 44, 63, 64, 65, 66	col. 27, line 66 to col. 28, line 7; col. 30, line 36

45, 46, 48, 67, 68, 69, 70, 73, 74	col. 28, line 54 to col. 29, line 2
49	col. 8, lines 30-32
47, 71, 72	col. 30, line 36
50, 51, 52, 53	col. 8, lines 34 to 35; Example 3
54	col. 7, line 64 to col. 8, line 17; Example 3
55, 62	col. 8, lines 12 to 33; Example 3
56, 57, 58, 59	col. 8, lines 12 to 16
60	Example 3
61	col 8, lines 33-40; Example 3
62	col. 8, lines 17-33; Example 3

Thus, new claim 42 is directed to a polypeptide comprising a designed zinc finger protein and at least one functional domain; claims 43 to 53 depend from claim 42 with added claims 44 to 49 further defining the functional domain and added claims 50 to 53 being directed to polynucleotides encoding fusions of designed zinc finger polypeptides and at least one function domain. Thus, added claims 42-53 are drawn to designed zinc finger proteins (or polynucleotides encoding these polypeptides) as described in the specification, for example at col. 8, lines 17 to 33; col. 27, line 66 to col. 28, line 7; col. 28, lines 54 to 60; and col. 30, lines 36 to 37. Applicants note that the term "designed zinc finger polypeptide" includes screened and/or selected zinc finger polypeptides, as described for example at col. 4, line 60 to col. 5, line 8. Added claims 42-53 are also related to the original claims in that they comprise a designed zinc finger polypeptide, for example as encoded by one or more members of the libraries of original claims 1-4, 9, 10, 19, 20, and 30 to 41 or as obtained by the methods of claims 5 to 8 and 11 to 13, with the additional element of at least one functional domain.

Added claims 54-74 are method claims directed to methods of altering expression of a chromosomal gene, as described for example, at col. 7, lines 64 to 67; col. 8, lines 12

to 17; and col. 30, lines 25 to 37 and in original method claims 5 to 8 and 14 to 18, with the added element that the designed zinc finger polypeptide that binds to the target sequence in the chromosomal gene modulates expression of the chromosomal gene. The Examples detail experiments wherein methods comprising a designed zinc finger and optional functional domain(s) were used to alter expression of a chromosomal gene.

Conclusion

Claims 1 to 74 are currently pending. Claims 1 to 41 were present in U.S. Patent No. 6,007,988 as issued. Claims 42 to 74 have been added herein. As can be seen from the foregoing, Applicants have not added any substantive issues to this reissue application and have only added claims that are supported by the original specification which details experiments using compositions comprising a designed zinc finger and a functional domain and methods of altering expression of a chromosomal gene. For the reasons detailed above, the added claims are also related to the original claims. Accordingly, the added claims do not depart from the invention as originally disclosed. It is respectfully submitted that the claims presented herein are described and enabled in the original patent specification.

Since the original patent issued on December 28, 1999, Applicants have timely filed a request for a broadening reissue and have complied with PTO rules governing reissue applications filed after November 7, 2000. The reissue claims are allowable for the same reasons that the original patent claims were so found and for the reasons set forth herein.

It is understood that there are no changes to be made to the drawings and that, pursuant to 37 C.F.R. 1.173 and M.P.E.P. § 1413, Applicants submit a clean copy of each drawing sheet of the printed patent herewith.

The Examiner is requested to contact Applicants' undersigned attorney if there are any questions regarding this application.

Respectfully submitted,

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Version Showing Changes Made to the Specification

The paragraph beginning at column 9, line 5 has been amended as follows:

To the best knowledge of the inventors, design of a zinc finger polypeptide and its successful use in modulation of gene expression (as described below) has never previously been demonstrated. This breakthrough presents numerous possibilities. In particular, zinc finger polypeptides could be designed for therapeutic and/or prophylactic use in regulating the expression of disease-associated genes. For example, zinc finger polypeptides could be used to inhibit the expression of foreign genes (e.g., the genes of bacterial or viral pathogens) in man or animals, or to modify the expression of mutated host genes (such as oncogenes).

The paragraph beginning at column 12, line 34 has been amended as follows:

Colonies were transferred from plates to 200 ml 2xTY/Zn/Tet (2xTY containing 50 μ M Zn(CH₃COO)₂ and 15 μ g/ml tetracycline) and grown overnight. Phage were purified from culture supernatant by two rounds of precipitation using 0.2 volumes of 20% PEG/2.5M NaCl containing 50 μ M Zn(CH₃COO)₂, and resuspended in zinc finger phage buffer (20 mM HEPES pH7.5, 50 mM NaCl, 1 mM MgCl₂ and 50 μ M Zn(CH₃COO)₂). Streptavidin-coated paramagnetic beads (Dynal) were washed in zinc finger phage buffer and blocked for 1 hour at room temperature with the same buffer made up to 6% in fat-free dried milk (Marvel). Selection of phase was over three rounds: in the first round, beads (1 mg) were saturated with biotinylated oligonucleotide (~80 nM) and then washed prior to phage binding, but in the second and third rounds 1.7 nM oligonucleotide and 5 μ g poly dGC (Sigma) were added to the beads with the phage. Binding reactions (1.5 ml) for 1 hour at 15°C. were in zinc finger phage buffer made up to 2% in fat-free dried milk (Marvel) and 1% in Tween 20, and typically containing 5x10¹¹ phage. Beads were washed 15 times with 1 ml of the same buffer. Phage were eluted by shaking in 0.1M triethylamine [triethylamine] for 5 min and neutralised with an equal volume of 1M Tris pH7.4. Log phase *E. coli* TG1 in 2xTY were infected with eluted phage for 30 min at 37°C. and plated as described above. Phage titres were determined by plating serial dilutions of the infected bacteria.

The paragraph beginning at column 19, line 62 has been amended as follows:

Flexible flat-bottomed 96-well microtitre plates (Falcon) were coated overnight at 4°C. with streptavidin (0.1 mg/ml in 0.1M NaHCO₃ pH8.6, 0.03% NaN₃). Wells were blocked for one hour with PBS/Zn (PBS, 50 μ M Zn (CH₃COO)₂) containing 2% fat-free dried milk (Marvel), washed 3 times with PBS/Zn containing 0.1% Tween, and another 3 times with PBS/Zn. The "bound" strand of each oligonucleotide library was made synthetically and the other strand extended from a 5'-biotinylated universal primer using

DNA polymerase I (Klenow fragment). Fill-in reactions were added to wells (0.8 pmole DNA library in each) in PBS/Zn for 15 minutes, then washed once with PBS/Zn containing 0.1% Tween, and once again with PBS/Zn. Overnight bacterial cultures each containing a selected zinc finger phage were grown in 2xTY containing 50 mM $\text{Zn}(\text{CH}_3\text{COO})_2$ and 15 $\mu\text{g/ml}$ tetracycline at 30°C. Culture supernatants containing phage were diluted tenfold by the addition of PBS/Zn containing 2% fat-free dried milk (Marvel), 1% Tween and 20 $\mu\text{g/ml}$ sonicated salmon sperm DNA. Diluted phage solutions (50 μl) were applied to wells and binding allowed to proceed for one hour at 20°C. Unbound phage were removed by washing [D] 5 times with PBS/Zn containing 1% Tween, and then 3 times with PBS/Zn. Bound phage were detected as described previously (Griffiths et al., 1994 EMBO J. [In Press] 13(14):3245-3260), or using HRP-conjugated anti-M13 IgG (Pharmacia), and quantitated using software package SOFT-MAX 2.32 (Molecular Devices Corp).

The paragraph beginning at column 21, line 61 has been amended as follows:

Table 2 summarises frequently observed amino acid-base contacts in interactions of selected zinc fingers with DNA. The given contacts comprise a "syllabic" recognition code for appropriate triplets. Cognate amino acids and their positions in the α -helix are entered in a matrix relating each base to each position of a triplet. Auxiliary amino acids from position +2 can enhance or modulate specificity of amino acids at position -1 and these are listed as pairs. Ser or Thr at position +6 permit Asp +2 of the following finger (denoted Asp ++2) to specify both G and T indirectly, and the pairs are listed. The specificity of Ser +3 for T and Thr +3 for C may be interchangeable in rare instances while Val +3 appears to be consistently ambiguous.

The paragraph beginning at column 29, line 55 has been amended as follows:

Immunofluorescence microscopy of transfected Ba/F3+p190 cells in the absence of IL-3 shows chromatin condensation and nuclear fragmentation into small apoptotic bodies, while the nuclei of Ba/F3+p210 cells remain intact. Northern blots of total cytoplasmic RNA from Ba/F3+p190 cells transiently transfected with the anti-BCR-ABL peptide revealed reduced levels of p190^{BCR-ABL} mRNA relative to untransfected cells. By contrast, similarly transfected Ba/F3+p210 cells showed no decrease in the levels of p210^{BCR-ABL} mRNA [(FIG. 12)]. The blots were performed as follows: 10 mg of total cytoplasmic RNA, from the cells indicated, was glyoxylated and fractionated in 1.4% agarose gels in 10 mM NaPO_4 buffer, pH 7.0. After electrophoresis the gel was blotted onto HYBOND-N (Amersham), UV-cross linked and hybridized to an ³²P-labelled c-ABL probe. Autoradiography was for 14 h at -70°C. Loading was monitored by reprobng the filters with a mouse [b-acting] β -actin cDNA.